A STUDY OF THE VARIATION WITHIN AND BETWEEN PROSTANTHERA MONTICOLA AND P. WALTERI (LABIATAE) USING LEAF VOLATILE OILS

by

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ABSTRACT

Conn, B. J. & Whiffin, T. A study of the variation within and between *Prostanthera monticola* and *P. walteri* (Labiatae) using leaf volatile oils. *Muelleria* 6(5): 375-382 (1987). — The leaf volatile oils showed a marked distinction between *Prostanthera monticola* and *P. walteri,* supporting the morphologically-based taxonomic conclusion that they represent two species. The pattern of variation within each species was found to reflect the isolated, perhaps relictual nature of the populations.

INTRODUCTION

Prostanthera monticola and *P. walteri* (together with *P. porcata*) occupy a taxonomically unique position within section *Klanderia.* All three species have larger leaves and slightly larger flowers than those of the other species in this section. Although most of the species of section *Klanderia* are confined to the lowland arid and semi-arid regions (as defined by Gentilli 1972), these three species occur in the mountainous subhumid region at altitudes above 450 metres. *Prostanthera monticola* and *P. walteri* are the only species of this section which occur above the snowline, at altitudes up to 1833 metres.

In a revision of *Prostanthera* section *Klanderia,* Conn (1984) recognized *Prostanthera monticola* as a species which was morphologically distinct from *P. walteri.* Prior to this, the name *P. walteri* had been collectively applied to these two taxa, even though Willis (1973) realized that *P. walteri* (*s.str.*) was, at least in part, distinct from other populations which were also referred to this species. The main morphological features which distinguish these two species are that *P. monticola* has prophylls which are 10-18 mm long (cf. 4-6.5 mm long in *P. walteri*) and has a hairy inner surface of the calyx-lobes (cf. glabrous in *P. walteri*).

Prostanthera monticola occurs in the Southern Tablelands of New South Wales and at Mount Buffalo in Victoria, whereas *P. walteri* occurs in East Gippsland of Victoria and at Mt Imlay (South Coast) in New South Wales (Fig. 1). The two species tend to occur as relatively small, isolated populations which are confined to mountain tops and ridges.

The purpose of this study was to examine the pattern of variation within and between the two species using leaf volatile oils. This information proved useful in two main ways. Firstly, it allowed an assessment of the extent to which these characters supported the morphologically-based distinction between the two species. Secondly, it provided information on the amount of differentiation within each species.

No other works on the leaf volatile oils of *Prostanthera,* except that of Conn (1984), have been published, although E. V. Lassak (pers. comm.) has investigated some species. Conn used leaf volatile oils to give a brief taxonomic evaluation of the infrageneric classification proposed by Bentham (1870) and to evaluate the patterns of geographic variation of *P. aspalathoides.*

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MATERIALS AND METHODS

Collections were made from two populations of *P. monticola* and four populations of *P. walteri,* representing as far as possible the distributional range within each species (Table 1 & Fig. 1). The other known populations of *P. monticola* (Fig. 1) could not be relocated. This species is apparently very rare (in New South Wales) and each population comprises a few scattered plants which are easily overlooked. Fresh foliage samples were collected separately from eight plants per population (except five from population W3), sealed in polyethylene bags and stored at approximately 2°C until processed. The voucher plant material which was

Table 1. Details of the two populations of *P. monticola* and four populations of *P. walteri* studied for leaf volatile oils.

Species	Population Code	Locality	Collection Numbers	Number of Specimens
P. monticola	M1	Crystal Brook Falls	Conn 1422A, 1422B-1428	8
”	M2	Middle Creek	Conn 1429-1432A, 1432B-1435	8
P. walteri	W1	Mt Elizabeth II	Conn 1405-1412	8
”	W2	Mt Ellery	Conn 1397-1404	8
”	W3	Mt Kaye	Forbes 1967-1971	5
”	W4	Mt Imlay	Conn 1388-1395	8

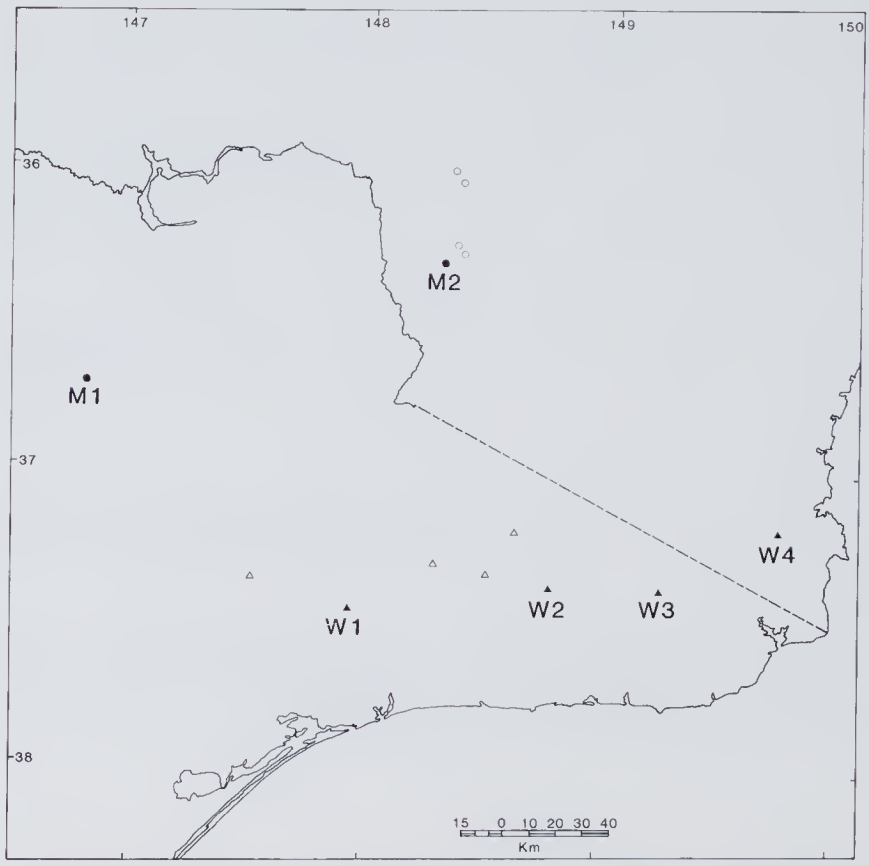


Fig. 1. Distribution map of *Prostanthera monticola* and *P. walteri. P. monticola* — ● populations sampled in this study; ○ other known populations. *P. walteri* — ▲ populations sampled; △ other known populations.

Table 2. Population means and ranges, with F-values for the twelve more important compounds (those for which the population mean was greater than 5% in one or more populations). All compounds are significant at the 0.001 level.

CHARACTERS	POPULATIONS						F-Value
	M1 Mean (Range)	M2 Mean (Range)	W1 Mean (Range)	W2 Mean (Range)	W3 Mean (Range)	W4 Mean (Range)	
8	42.341 (23.217-58.246)	9.368 (4.142-19.650)	0.139 (0.003-0.296)	0.102 (0.013-0.311)	0.389 (0.180-0.666)	0.543 (0.111-1.561)	90.497
13	0.382 (0.021-1.886)	0.925 (0.353-2.205)	0.873 (0.248-2.887)	6.591 (1.086-12.840)	4.300 (0.510-9.235)	1.449 (0.459-4.834)	9.083
20	10.886 (4.538-13.992)	13.319 (7.676-23.241)	15.716 (8.796-21.635)	10.514 (5.516-21.802)	4.913 (2.166-7.098)	7.559 (5.072-10.968)	6.741
22	2.308 (0.231-9.084)	7.119 (5.192-11.370)	0.412 (0.200-0.893)	0.733 (0.515-1.050)	0.470 (0.310-0.793)	0.201 (0.003-0.760)	26.726
29	0.477 (0.183-0.805)	1.244 (0.927-1.568)	1.660 (1.306-2.218)	1.063 (0.570-2.271)	6.089 (2.492-12.812)	2.070 (0.246-6.868)	5.661
33	2.405 (0.050-5.705)	5.235 (2.871-6.407)	0.163 (0.080-0.378)	0.319 (0.148-0.479)	0.137 (0.003-0.285)	0.193 (0.121-0.290)	34.758
38	3.898 (1.606-5.527)	6.321 (3.957-7.862)	0.422 (0.137-0.699)	1.451 (0.003-5.412)	0.256 (0.003-0.951)	0.688 (0.003-1.525)	36.681
56	2.066 (0.722-4.572)	2.116 (0.003-3.561)	5.405 (1.811-8.473)	17.287 (6.738-24.141)	5.512 (2.179-10.765)	2.943 (0.809-6.788)	26.665
60	0.519 (0.069-2.338)	3.644 (0.627-7.570)	6.069 (2.095-12.238)	2.170 (0.699-3.655)	1.202 (0.332-1.746)	9.437 (2.162-24.656)	6.353
65	0.212 (0.072-0.516)	0.278 (0.003-0.474)	5.747 (1.604-14.481)	10.579 (5.603-20.051)	6.388 (2.893-15.895)	4.434 (2.122-7.899)	11.427
75	1.378 (0.243-3.372)	0.389 (0.111-0.939)	12.737 (4.566-23.484)	17.423 (4.419-31.359)	14.138 (5.360-22.457)	9.673 (3.580-15.326)	10.345
85	0.093 (0.003-0.335)	0.040 (0.003-0.266)	0.317 (0.003-0.895)	0.112 (0.003-0.466)	6.069 (1.182-11.752)	4.793 (2.102-11.611)	11.294

collected from each individual is lodged at the National Herbarium of Victoria (MEL).

The leaves were steam distilled and the volatile oils were recovered according to the methods detailed by Whiffin (1982) and Newnham *et al.* (1986). The oils were analyzed by gas chromatography, with the percentage composition determined using the methods of Newnham *et al.* (1986).

The data were subjected to a one-way analysis of variance (ANOVA) and Student-Newman-Keuls (SNK) multiple range test to determine, respectively, which compounds showed significant differences between populations, and which populations were involved. Population means of selected significant compounds were submitted to contour mapping to illustrate the pattern of variation present within these characters.

Multivariate analyses involved submitting the Manhattan Metric distance matrix between individuals and, separately, that between population means, to clustering, ordination and minimum spanning tree procedures. The analyses included: clustering by use of the fusion criteria of unweighted pair-group method using averages (UPGMA) and weighted pair-group method using averages (WPGMA); ordination by principal coordinates analysis (PCDA); and computation of a minimum spanning tree. Where different analyses produced essentially similar results, only one set of results is presented here.

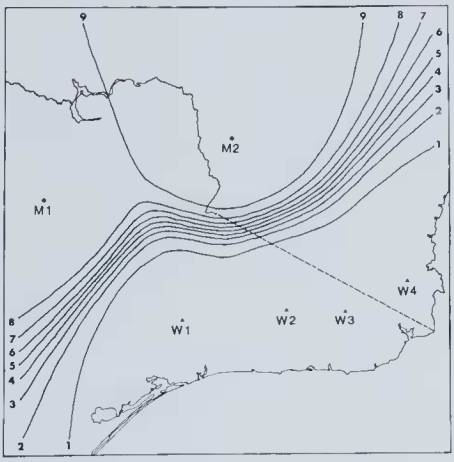
The rationale for the use of these various univariate and multivariate techniques in the study of variation is provided by Whiffin (1982). The methods and computer programs used here are those detailed by Whiffin (1982), except that multivariate techniques were undertaken using the NT-SYS program package (Rohlf 1985).

RESULTS

A total of 191 compounds were detected within the volatile oils of the two species. The results relating to the twelve more important compounds (those for which the population mean was greater than 5% in one or more populations) are presented in Table 2. In the analysis of variance, 136 of these 191 compounds were significant at the 0.05 level, of which 104 were also significant at the 0.01 level. In fact, 78 compounds were significant at the 0.001 level. In the results from the SNK test, which is generally a more robust test (Adams & Turner 1970), 95 compounds showed significant differences between populations at the 0.01 level.

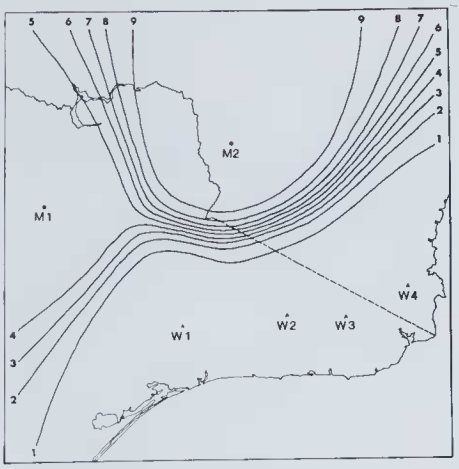
A study of the significant compounds by contour mapping and an examination of their SNK test results showed that they could be placed into one of two groups, each representing a different major pattern of variation. The first group showed a pattern of variation involving a distinction between the two populations of *P. monticola* and the four populations of *P. walteri*. An example of this pattern is found in compound 43 (Table 2 & Fig. 2). Included in this group are a number of compounds which also show a significant difference between the two populations of *P. monticola*, in addition to the significant difference between the two species. Examples of this latter pattern include many of the more important, and generally more highly significant compounds, such as compounds 8, 33 and 38 (Table 2 & Fig. 3). Some other compounds show this basic pattern, although they exhibit a slight overlap between the two species in the SNK test. Examples of this pattern include compounds 13, 65 and 75 (Table 2).

The second group of compounds show a pattern of variation involving a significant difference between one population and all other populations, of either species. Examples of this pattern include compound 56 (Table 2 & Fig. 4), which shows population W2 as being significantly distinct from all others, and compound 29 (Table 2 & Fig. 5) which shows population W3 as being significantly distinct from all others. Within the 95 compounds that have significant SNK tests, examples can be found where each of the six populations in turn show a significant difference from the other five together. The more common patterns involve the distinction of populations M2 or W4.



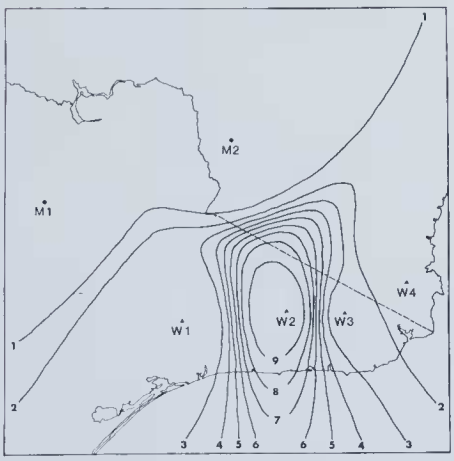
M2 M1 W3 W1 W4 W2

Fig. 2. Contour map (with summary of SNK test) of percentage composition of compound 43 for populations of *P. monticola* and *P. walteri*. Contour values are: 1 = 0.154, 2 = 0.407, 3 = 0.660, 4 = 0.913, 5 = 1.166, 6 = 1.413, 7 = 1.672, 8 = 1.925, 9 = 2.178.



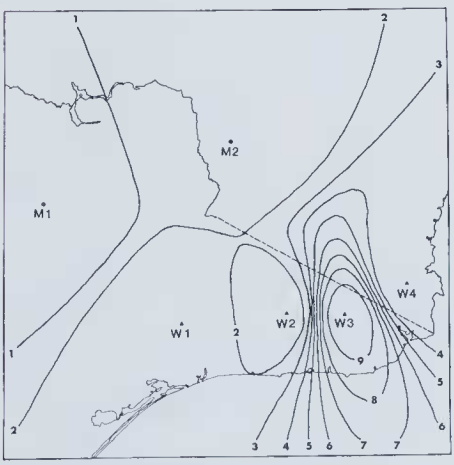
M2 M1 W2 W4 W1 W3

Fig. 3. Contour map (with summary of SNK test) of percentage composition of compound 33 for populations of *P. monticola* and *P. walteri*. Contour values are: 1 = 0.421, 2 = 0.987, 3 = 1.553, 4 = 2.120, 5 = 2.686, 6 = 3.253, 7 = 3.819, 8 = 4.385, 9 = 4.952.



W2 W3 W1 W4 M2 M1

Fig. 4. Contour map (with summary of SNK test) of percentage composition of compound 56 for populations of *P. monticola* and *P. walteri*. Contour values are: 1 = 2.910, 2 = 4.599, 3 = 6.288, 4 = 7.976, 5 = 9.665, 6 = 11.354, 7 = 13.042, 8 = 14.731, 9 = 16.420.



W3 W4 W1 M2 W2 M1

Fig. 5. Contour map (with summary of SNK test) of percentage composition of compound 29 for populations of *P. monticola* and *P. walteri*. Contour values are: 1 = 0.788, 2 = 1.410, 3 = 2.032, 4 = 2.655, 5 = 3.277, 6 = 3.899, 7 = 4.521, 8 = 5.143, 9 = 5.766.

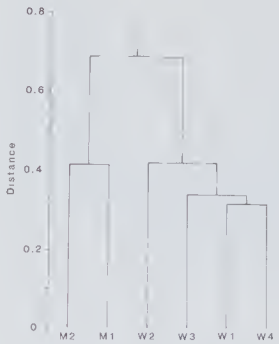


Fig. 6. Dendrogram from the cluster analysis (unweighted pair-group method) of the populations of *P. monticola* and *P. walteri* based on leaf volatile oil composition.

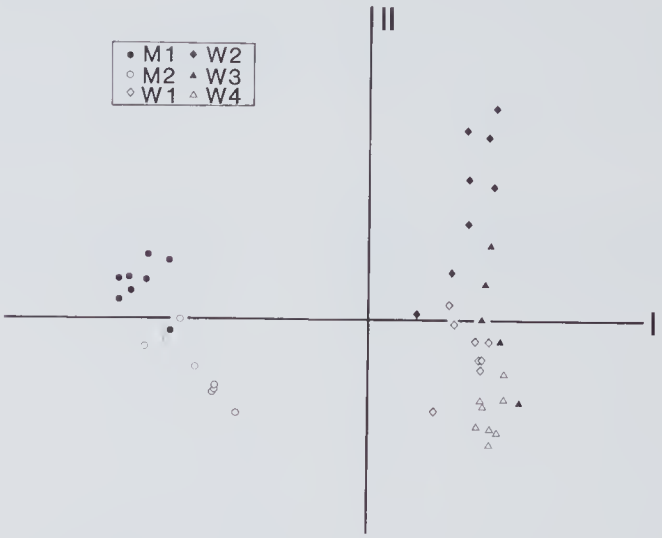


Fig. 7. Principal coordinates analysis ordination plot of the individuals of *P. monticola* and *P. walteri* based on leaf volatile oil composition (axis 1 accounts for 46% of the total variation and axis 2 for 14%).

These two major patterns of variation account for most of the more important or more highly significant compounds. Thus, of the twenty more highly significant compounds (with an F-value greater than 20.0 in the analysis of variance), six show a significant difference between the two populations of *P. monticola* and the four populations of *P. walteri* (cf. Fig. 2). A further three show a significant difference between the two populations of *P. monticola* (cf. Fig. 3), as well as a significant difference between the species. Five more compounds show this basic pattern of distinction between the two species, but with a slight overlap between the two. Within the remaining six more important compounds, five show the second major pattern of variation, distinguishing one population from all others, variously involving populations M2, W2 or W4 (cf. Figs 4 & 5). The last compound distinguishes W3 and W4 from each other, and from the other four populations together.

Of the twelve more important compounds (Table 2), three (compounds 8, 33 and 38) distinguish M1 from M2, and from all other populations. A further three (compounds 13, 65 and 75) distinguish the two species, although with a slight degree of overlap. Three compounds distinguish one population from all others, involving population M2 (compound 22), population W2 (compound 56), and population W3 (compound 29). Of the remaining compounds, one distinguishes

populations W3 and W4 together from all other populations (compound 85), whereas the other two provide a less distinct separation of individual populations (compounds 20 and 60).

The remaining significant compounds, those not showing one of the two major patterns of variation, show various patterns. However, most can be related back to one of the two major patterns, but with greater overlap between the distinguished groups.

The cluster analysis of the population means (Fig. 6) shows that there is a clear distinction between the two species, whereas within each species the populations are relatively distinct. The results from the other multivariate analyses of these data (ordination and minimum spanning tree) provided essentially the same results.

In the multivariate analyses of the individuals, the clustering, ordination and minimum spanning tree analyses are similar. As the ordination results are visually easier to comprehend, they are presented here (Fig. 7). The first two axes of the principal coordinates analysis provide a useful simplification of the data because they account for 59.47% of the total variation. The ordination on these axes produces distinct clusters. The major distinction, on axis 1, is between the two populations of *P. monticola* and the four populations of *P. walteri.* Variation within *P. walteri* was projected mainly on axis 2, whereas that within *P. monticola* was projected mainly on axis 3. The various populations within each species are relatively homogeneous, with the individuals of a given population grouping together in the ordination plot (and cluster dendrogram), although there is some overlap of the various population clusters.

DISCUSSION

The results from all analyses undertaken, both univariate and multivariate, indicate a basic similarity between the two species on leaf volatile oil composition, concordant with a common ancestry for the two, but with sufficient quantitative differences to confirm their recognition as distinct species. This distinction between the two species, based on the volatile oils, confirms the conclusions drawn from the morphological data (Conn 1984).

The patterns of variation observed, within and between populations, may reflect the separate, perhaps relictual, nature of the six populations. The individuals within any one population showed a tendency to group together, separate from other populations, in the multivariate analyses (eg. ordination, Fig. 7). Paralleling this, in the SNK tests, each population was significantly different from all others for at least one compound. This suggests that there has been separate evolution, relating to selection or genetic drift, within each population, with little or no gene flow between the populations, at least in recent times.

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